**Agarose Gel Electrophoresis of Double Digestion of pKan and pAmp**

**Abstract**

This experiment was designed in order to double digest both pAmp and pKan plasmids using restriction enzymes. A double digest is when two restriction enzymes are used to separate DNA into two fragments. Restriction enzymes are a way for bacteria to “cut” and destroy DNA from an invading virus. A gel electrophoresis will be run to analyze the results we get. The gel electrophoresis is an appropriate tool used here because it will allow us to see the sizes of the fragments made by the double digest. The DNA that we used are DNA plasmids known as pAmp and pKan. The restriction enzymes used are called HindIII and BamHI. The experiment turned out to be a moderate success. The gel showed results that indicated that a double digest had indeed occurred. However, there were a few errors.

**Introduction and Background**

When they were discovered in the 1950s, restriction enzymes, or endonucleases, revolutionized the world of molecular biology. [1] This allowed scientists to reach new levels of genetic engineering and recombinant gene technology. Restriction enzymes are enzymes that are produced and used in bacteria to fight off invading viruses. They do this by digesting or “cutting” the virus DNA at a specific point. Cutting the DNA renders the bacteriophage useless and the bacteria safe. The practical uses of this are huge in the field of recombinant DNA and genetic engineering. Scientist can, with plenty of endonuclease options, cut DNA sequences out of organisms with extreme accuracy and specificity. [2]

Of course, sometimes using restriction enzymes requires analysis. This is where the helpful tool of gel electrophoresis comes in. The purpose of gel electrophoresis is to separate DNA based on fragment size. The DNA is placed in wells in the gel and an electric charge is applied. The negative charge of DNA causes it to migrate towards the positive anode; the gel provides a matrix that allows smaller fragments to move further, and slows the larger fragments. The varying movement speeds creates bands that can be analyzed to show size of fragments. [3]

In this lab, we performed a double digestion on the DNA of pAmp and pKan. This simply means two restriction enzymes are used on one DNA. The restriction enzymes we used are HindIII and BamHI. Once digested, a gel electrophoresis will be performed. The results will allow analysis on whether the DNA was digested correctly.

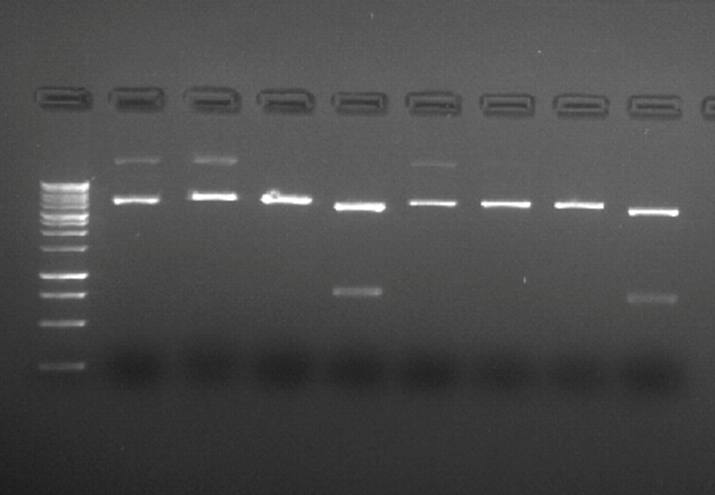
**Materials and Method**

The first step in this process was to prepare the gel. First, a 1x buffer was made. We started with a 50x TAE buffer, so to dilute it, we mixed 20mL of the TAE buffer with 980mL of distilled water to make 1000mL of 1x TAE buffer. 200 mL of that buffer was used to make the gel. We mixed 2g of agarose with 200mL of TAE buffer in a flask to make the gel solution. This solution was heated in the microwave for 1 minute and stirred, then placed in the microwave for 15 second intervals until it was dissolved. Then 2uL of ethidium bromide (Bio-Rad, Cat # 1610433) was added. Once it had cooled slightly, the gel solution was added to the casting tray, the comb was placed in it, and it was allowed to harden. Once the gel cooled and solidified, it was placed into the gel electrophoresis apparatus (Bio-Rad Cat # 1704468); the remaining 800mL of TAE buffer was poured into the tray until the gel was fully submerged. This completed the preparation of the gel electrophoresis apparatus.

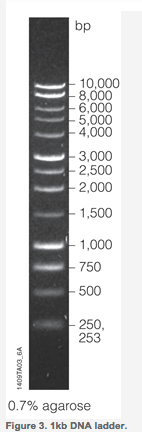
The next step in the process was to prepare the DNA samples that were to be used. Each partner used a different DNA plasmid: one used pAmp (Carolina Biologicals, Cat #211431**)** and one used pKan (Carolina Biologicals, Cat #211441). To simplify, the following describes one partner’s DNA preparation. The first sample was an uncut plasmid. In a micro centrifuge tube, 3uL of uncut pAmp, 2uL of 10x buffer, and 15uL of water were mixed to prepare the sample. Next was the single digest by HindIII (Thermo Fisher Scientific, Cat # FD0504). In a micro centrifuge tube, 1uL of HindIII, 3uL of pAmp DNA, 2uL of 10x buffer, and 14uL of water were added and mixed. Then, single digest by BamHI (Thermo Fisher Scientific, Cat # FD0054) was prepared by mixing 1uL of BamHI, 3uL of pAmp DNA, 2uL of 10x buffer, and 14uL in a micro centrifuge tube. The double digest was prepared by adding 1uL of both HindIII and BamHI, 3uL of pAmp DNA, 2uL of 10x buffer, and 13uL of water to a microcentrifuge tube. All samples should be gently mixed up and down. Next, all the samples were place in the incubator at 67°C for 15 minutes. Once out of the incubator, add 2uL of loading dye to each sample.

Now all that all the samples were prepared, the next step was to load them into the gel. First, 5uL of the ladder sample was added to the very left well. Finally, 22uL (all) of all the samples were then added to their respective wells. The order from left to right was the uncut 1, HindIII 1, BamHI 1, double digest 1, uncut 2, HindIII 2, BamHI 2, double digest 2. The electrodes were added to the appropriate sides and the gel ran for about 30 minutes. After completed, the gel was photographed using UV lighting to bring out the bands.

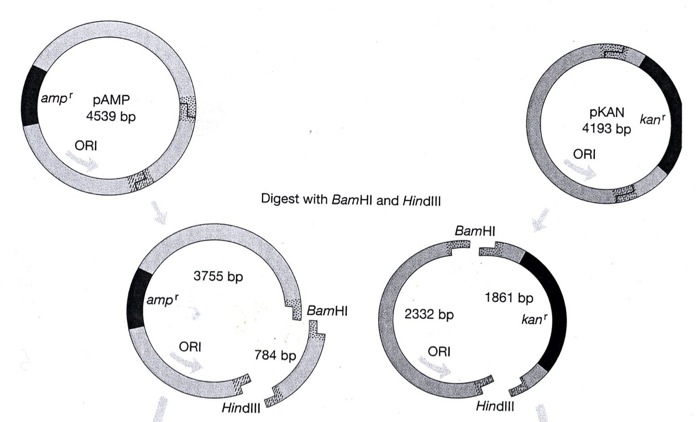
**Results and Analysis**



**Figure 1:** This is the UV picture of the gel electrophoresis that we ran.  From the left to right, the first is the ladder, the uncut 1, HindIII 1, BamHI 1, double digest 1, uncut 2, HindIII 2, BamHI 2, double digest 2.



**Figure 2**: This is the manufacturer’s ladder for a 0.7% agarose gel.



**Figure 3**: Single and double digests of pKan and pAmp with base pair numbers.

Refer to Figure 1 for results.

Both partners prepared samples of uncut DNA, DNA cut by HindIII and BamHI separately, and a double digest with them together.  The results appear to be mixed.  Wells 2 and 6 contained only uncut plasmid DNA, therefore should only have one band; however, there is clearly two bands.  This could be a result of many plasmids that had formed together to form the ghost well.

Wells 5 and 9 appear to be successful at first glance.  These two wells contained both HindIII and BamHI, and should have generated two bands. It is good that the higher band is brighter because that indicates double digestion. However, the problem is that 5 and 6 both have equal bands, but they’re supposed to have different DNA and therefore not look identical.

So, since both partners used the same plasmid, it must be determined which plasmid was used. Using the manufacturer ladder (Figure 2) here is key. From Figure 3, the double digest of pAmp creates a 3755 bp fragment and a 784 bp fragment. This lines up perfectly with both the double digest bands, suggesting that pAmp was used in all the digestions.

Wells 3 and 4, with HindIII and BamHI respectively, ran quite nicely. They both did not run quite as far as uncut pAmp. This is good because the linear digested DNA should not travel as far as the circular, more condensed uncut DNA.

The ladder also provided mixed results. The bands came out to be strong and bright, but the bands at the top are hard to discern and they seem blurry. Individual distinct bands are what are desired here. So, the ladder is fairly accurate, but not as accurate as desired. The quality of this ladder also indicates overall quality of the gel. That is to, say adequate, but not exceptional.

**Discussion/Conclusions**

This experiment centered around double digesting two different plasmids. The plasmids used were pAmp and pKan; the restriction enzymes used were HindIII and BamHI. Samples were created not only for the double digests, but also single digests and uncut plasmids. These were used as points of reference. We then used gel electrophoresis for the analysis of the digestion. The bands in the gel were able to tell us that we did indeed digest plasmid DNA.

Overall, this lab was a success. However, there were definitely some errors that interfered with our data. The major error in this lab was the use of the same DNA for both the partners. The protocol called for use of pKan and pAmp, but the bands suggest pAmp was used for all digestions. In the future it would be wise to make sure that both partners had correctly labeled tubes of different plasmids. Another error was not letting the gel run long enough. Allowing the gel to run longer in the future will create clearer and more distinct bands in the ladder.

Since this lab was a success, in terms of creating double digested plasmids, future experiments could involve insertion of that cut DNA into bacteria. So, the pAmp fragment that was cut can be put into bacteria and then tests would be run to determine if it was a successful recombination or not.

**Bibliography**

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3. Lee, P.Y., et al., *Agarose gel electrophoresis for the separation of DNA fragments.* J Vis Exp, 2012(62).